Journal of Kerbala for Agricultural Sciences Issue (2), Volume (12), (2025)

Herbicidal and allelopathic effects on seed germination and growth of annual ryegrass and celery plants

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https://doi.org/ 10.59658/jkas.v12i2.2683

Received: Dec. 24, 2024

Accepted: Mar. 19, 2025

Published: June 20, 2025

Abstract

In response to environmental concerns and human health, the global demand is focused on decreasing the use of synthetic herbicides and increasing the application of allelopathic plant extracts. This study aims to investigate the effects of various concentrations of synthetic herbicides (HC) (0, 1, 2, 3, and 4 mL/L) and laurel leaf extract (LLE) (0, 2.5, 5, 10, and 15%) on annual ryegrass (ARG) and celery plants. The results revealed that manipulating these concentrations significantly impacts the seed growth characteristics of both plants. Results indicated that applying HC significantly influenced all attributes in this study, recording the minimum value for ARG plants among all treatments. Moreover, the germination index (12.72 days), germination speed (12.64 days), and germination energy (52.67%) of celery seeds were stimulated by LLE. LLE allelopathic applications enhanced the coefficient of velocity for ARG seeds. Comparing HC and LLE, HC has a greater effect, decreasing seed germination across all seed parameters. Additionally, when comparing doses between HC and LLE, all doses of HC inhibited seed ARG to zero for all characteristics. Untreated celery seeds improved and significantly influenced priority attributes. This approach demonstrates the potential for reducing the environmental footprint of traditional herbicides, offering a more sustainable alternative for weed management and crop protection. It provides a foundation for researchers and farmers to explore alternative doses and methods for allelopathic extraction and plant applications.

Keywords: *Lolium rigidum*, *Apium graveolens*, Synthetic Application, Laurel Extract, Allelochemicals, Inhibitions

Introduction

One of the main challenges facing the agricultural sector in the 21st century is the production of sufficient food to meet the increasing needs of a rapidly growing population while preserving the ecosystem and protecting the socio-economic well-being of food producers and rural communities. In agricultural systems, competition from weeds is one of the main factors that reduce the yield of field crops, including vegetables [1]. Intensive farming practices, climate change, and natural disasters affect weed dynamics, necessitating changes in management practices [2]. Chemical weed control is a reliable and straightforward choice for managing weeds. However, reliance on herbicides alone is not encouraged due to their environmental impact and the development of herbicide-resistant weeds [3]. Various strategies have been explored to manage weeds effectively while minimizing the negative environmental and human health impacts [4, 5]. Integrated weed management (IWM) is a system approach to maintain the weed population below the economic threshold level [6].

Vegetables have a significant impact on human health by offering essential nutritional value, vitamins, and minerals [7]. Vegetables are particularly vulnerable to weed competition during their initial growth period, with weeds causing 70 to 80 percent yield reductions. Thus, controlling weeds during the critical weed-free period is essential [3]. Intercropping leafy vegetables with weeds poses competition for resources. If all weeds in crops were controlled, global food production would be 10-25% higher [1]. One such significant weed is annual ryegrass (ARG), scientifically named *Lolium rigidum*. It competes with crops for nitrogen, light, and soil nutrients, leading to lower biomass and reduced yields [8]. Surveys have identified ARG as one of the most common weed species in cropping fields [9]. ARG competes with crops leading to lower biomass and reduced yields for the crop [10]. Many field vegetables are weak competitors against weeds, leading to high costs for weed management [11].

ARG can be outcompete with various vegetables, including celery (*Apium graveolens* L.) of the Apiaceae family. Celery is a leafy vegetable that grows in cool climates and is sensitive to high temperatures. It is important due to its nutritional value and medicinal properties [12]. However, ARG is a significant weed in celery fields, and its control is crucial for maintaining crop yields and quality [13]. The development of allelopathy has led to new methods of weed control [1]. Plant extracts from bay laurel (*Laurus nobilis*) have demonstrated significant potential in weed control, presenting sustainable and eco-friendly alternatives to synthetic herbicides [14]. Research indicates that these natural extracts contain bioactive compounds that inhibit weed growth effectively. The allelopathic properties of bay laurel (LLE), which involve the release of chemicals that suppress neighboring plant growth, are particularly advantageous [15]. By integrating bay laurel extracts into weed management programs, farmers can achieve effective weed control while promoting biodiversity and soil health [16]. This study assessed the effects of herbicides (HC) and laurel leaf extract (LLE) concentrations on ARG and celery plants to determine optimal doses, aiming to reduce synthetic HC use sustainably and minimize inter-cropping competition between ARG and celery. This approach enhances crop yields and also contributes to the long-term sustainability of agricultural ecosystems.

Materials and Methods

Preparation of Aqueous Extract

The aqueous leaf extracts of the air-dried bay laurel plant (LLE), scientifically known as *Laurus nobilis* and belonging to the Lauraceae family, were prepared by mixing 10 g of leaves powder with 100 mL of distilled water. The samples were then placed in a shaker machine for improved homogenization and allowed to undergo overnight agitation. The leaf extracts were filtered using filter paper and then subjected to centrifugation at 1000 rpm for ten minutes. Subsequently, the supernatant was filtered through a micropore filter with a pore size of 0.45 µm. Ultimately, the resulting extract was acquired and preserved in a refrigerator at a temperature of 4°C until needed. The bay laurel leaves content was summarized (Table 1).

Table (1): Ingredients of *Laurus nobilis* leaf plant applied in this study

Components	Composition (%)
1,8-cineole	54.44
Sabinene	9.03
***α*-terpinyl acetate**	10.78
***α*-pinene**	4.54
***Β*-pinene**	3.56
p-cymene	2.12
Terpinen-4-ol	2.63
***α*-terpineol**	3.09
γ-terpinene	0.67
Myrcene	0.55
Eugenol	0.35
Linalool	1.1
Eremanthin	0.31
Spathulenol	0.49
β-elemene	2.54
α-Phellandrene	0.6
Camphene	0.3

Experimental Material and Growing Conditions

The experiment was carried out during the gro0wing season at the Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq (35º 53' N, 45º 36' E). Before treating the seeds of both plants with HC and LLE to avoid contamination, the seeds were sterilized with 10% sodium hypochlorite and then washed and rinsed three times with distilled water. The weed plant ARG (Figure 1) and celery seeds were soaked in different concentrations of HC solution (0, 1, 2, 3, and 4 mL/L) (Figure 2) and LLE allelopathy (0, 2.5, 5, 10, 15%) for 2 hours with gentle shaking to avoid seed damage. For this experiment, 20 seeds of each plant were placed in separate Petri dishes with a 9 cm diameter, and filter paper (Whatman® Number One) was placed under the seeds. Additionally, control plant seeds were used, treated only with deionized water without HC and LLE application. The experiment was composed of three replications of each seed sample, including control and test treatments. During the experiment, the Petri dishes were placed in a growth chamber incubated at 22°C. The germination process continued for 14 days. HC was supplied by Syngenta® Enterprise (Basel, Switzerland) under the commercial name TOPIK 100 EC, which is a concentrate containing 100 g of clodinafop-propargyl and cloquintocet-mexyl.

Figure (1): All parts and life cycle of ARG plant from seed to mature plant

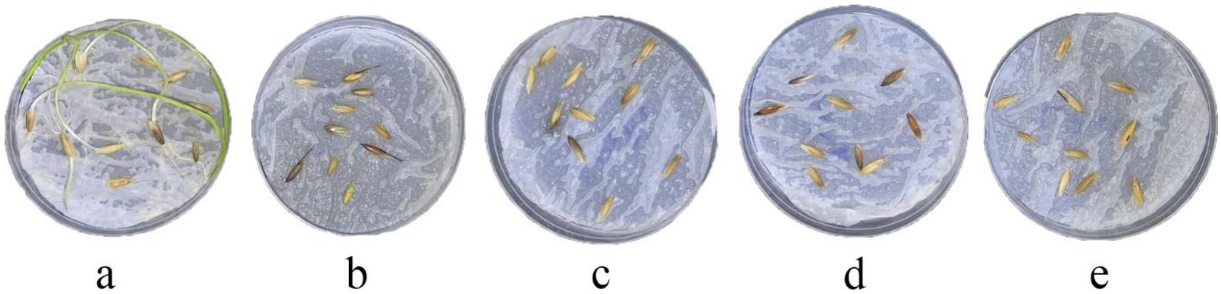

Figure (2): Responses of ARG seeds to HC solution at different concentrations: control (0 mL/L) (a), 1 mL/L (b), 2 mL/L (c), 3 mL/L (d), and 4 mL/L (e). Over 14 days, only the seeds germinated under untreated conditions.

Seed Analysis

During this study, several parameters were measured using different equations. Seed germination was calculated as an equation 1 [17].

$$\text{SG (\%)} = \frac{\text{Number of total germinated seeds}}{\text{Total number of seeds tested}} \times 100 \qquad \text{Eq. 1}$$

The germination time 50% (T_{50}) of the final germination rate was calculated by equation 2 [18]:

$$\text{T50} = \text{ti} \frac{(\frac{N}{2} - ni)(tj - ti)}{nj - ni} \qquad \text{Eq. 2}$$

Where N is the final number of germinated seeds, ni and nj represent the cumulative number of germinated seeds at times ti and tj, respectively, when

$ni < \frac{N}{2} < nj.$

Mean daily germination (MDG) can be calculated using the equation 3 [19]:

$$\mathrm{MDG} = \frac{\text{Germination percentage (SG \%)}}{\text{Total number of days}} \times 100 \qquad \text{Eq. 3}$$

Mean germination time (MGT) was measured by the equation 4 [17]:

$$\mathrm{MGT} = \frac{\Sigma \mathrm{Dn}}{\Sigma \mathrm{n}} \qquad \text{Eq. 4}$$

Where *n* is the number of germinated seeds on day *D*, and *D* is the number of days counted from the start of germination.

The Germination index (GI) was calculated by counting the germination rate at the first period and final period, according to equation 5 [20].

$$\mathrm{GI} = \frac{\text{No. of germinated seeds at first count}}{\text{Days of first count}} + \frac{\text{No. of germinated seeds at final count}}{\text{Days of final count}} \qquad \text{Eq. 5}$$

The Germination speed (GS) was calculated by Equation 6 as described by [21, 22].

$$\mathrm{GS} = \frac{\mathrm{N1}}{\mathrm{T1}} + \frac{\mathrm{N2}}{\mathrm{T2}} + \frac{\mathrm{N3}}{\mathrm{T3}} + \cdots + \frac{\mathrm{NK}}{\mathrm{TK}} \qquad \text{Eq. 6}$$

Let *N* be the number of seeds germinated on a given day, and *T* be the day number. GS is Germination Speed. N is the number of seeds germinated on a given day. T is the day number. NK is the total number of seeds germinated at the end of the period. TK is the number of days taken to reach the total germination.

The germination energy (GE) was calculated according to equation 7 [20].

$$\mathrm{GE}\ (\%) = \frac{\text{No. of germinated seeds of the 14 days}}{\text{Total number of seeds}} \times 100 \qquad \text{Eq. 7}$$

The coefficient of Velocity (CV) was calculated by Equation 8 [17]:

$$\mathrm{CV} = \frac{1}{\mathrm{MGT}} \times 100 \qquad \text{Eq. 8}$$

Statistical Analysis

The data were analyzed using a one-way analysis of variance (ANOVA) with a Completely Randomized Design (CRD) using XLSTAT statistical analysis software version 2019.2.2. Mean differences were assessed using Duncan's new multiple-range test with a significance level of $p \leq 0.05$. In addition, a multiple correlation test was conducted.

Results and Discussion

This study assessed the effects of optimal doses of herbicidal and allelopathic extracts on several variables of weed and vegetable plants. HC and allelopathy applications are crucial in managing seed germination and growth of weeds and vegetable crops. HCs are synthetic chemicals targeting specific physiological processes in weeds. Conversely, allelopathy involves natural plant chemicals (allelochemicals) that inhibit or delay germination and growth through biochemical interactions. In this study, we applied HC and allelopathic LLE to determine their effects on weed ARG and celery plants.

Response of Plants to Herbicidal and Allelopathic Weed Controls

The data (Figure 3) represented the impact of synthetic HC and allelopathic LLE application on the growth of ARG and celery. The statistical analysis conducted on the measurements indicated that the treatments affected the seed variables of both plants. The ARG seeds showed a decrease in seed germination, germination time 50%, mean daily germination and mean germination time to the minimum value (10.33%, 0.42 days, 0.74%, and 1.73 days, respectively) after exposure to synthetic HC applications (Figure 3a, b, c, d). The celery seeds exhibited the highest level of growth when they were immersed in a solution containing HC. This growth included seed germination (72.40%), germination time 50% (6.52 days), mean daily germination (5.17%), and mean germination time (9.79 days).

Despite the germination index and speed, the treatments were effective in both attributes, and different germination rates were found depending on the treatment (Figure 3e, f). Soaking the seeds of celery in LLE hastened germination to the maximum level (12.72 days and 12.64 days, respectively), while germination was the slowest in ARG seeds for germination index (1.50 days) and germination speed (1.49 days) when were immersed in HC. Additionally, the applications affected the germination energy percentage. The LLE application resulted in the highest energy percentage for celery seeds, reaching 52.67%, while the HC treatment resulted in germination energy of only 11.33% for ARG seeds (Figure 3g). The treatments significantly enhanced the coefficient of velocity during the germination period (Figure 3h). The LLE application resulted in the highest mean of 11.34%, while the lowest mean of 2.31% was observed in the HC treatment for ARG seeds. Based on these data, the influential actions and decreased seed germination and performance under synthetic HC application, followed by allelopathic LLE actions on the uncultivated weed plant ARG, were observed. Both applications had less effect on celery plant production, indicating that this approach has the potential to rely on natural resources for managing weed control.

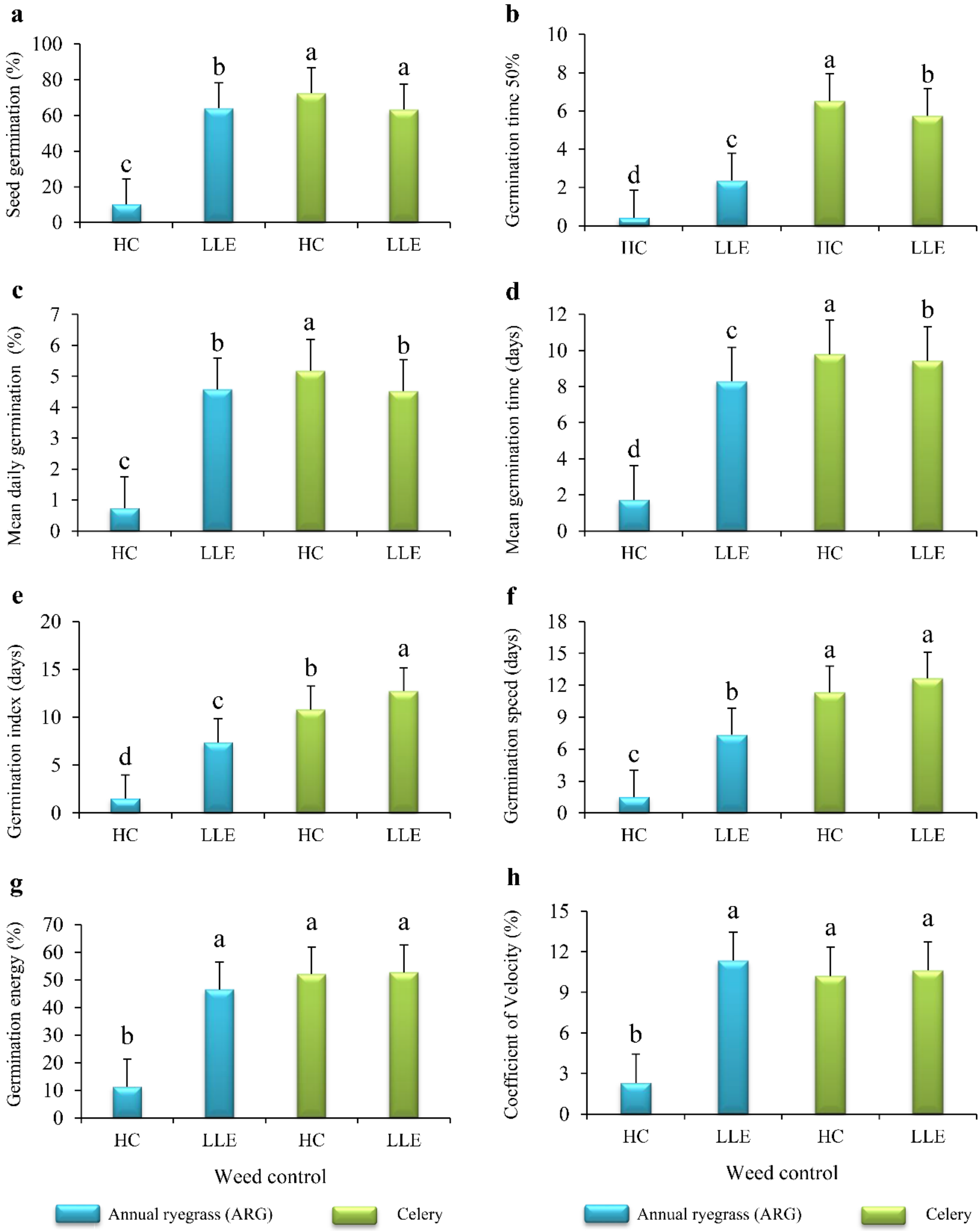

Figure (3): Responses of ARG and celery seeds to studied variables for synthetic HC and allelopathic LLE applications under various doses. According to Duncan's test, different letters indicate a statistical difference ($p \leq 0.05$) between treatments, and error bars represent standard deviations (n=3).

Effective Herbicidal and Allelopathic Weed Controls on the Seed Plants

According to the data (Figure 4) explained which of the herbicidal and allelopathic weed controls more significantly influenced the seed characteristics, contributing significantly ($p \leq 0.05$) to seed germination under HC and LLE. According to the data, it was found that HC was more effective than LLE on the studied variables and recorded the minimum results regarding seed germination (41.36%), germination time 50% (3.47 days), mean daily germination (2.96%), mean germination time (5.76 days), germination index (6.14 days), germination speed (6.40 days), germination energy (31.67%), and coefficient of velocity (6.26%). Conversely, LLE reached maximum values in the mentioned variables (63.60%, 4.05 days, 4.54%, 8.86 days, 10.05 days, 10.00 days, 49.60%, and 10.98%, respectively).

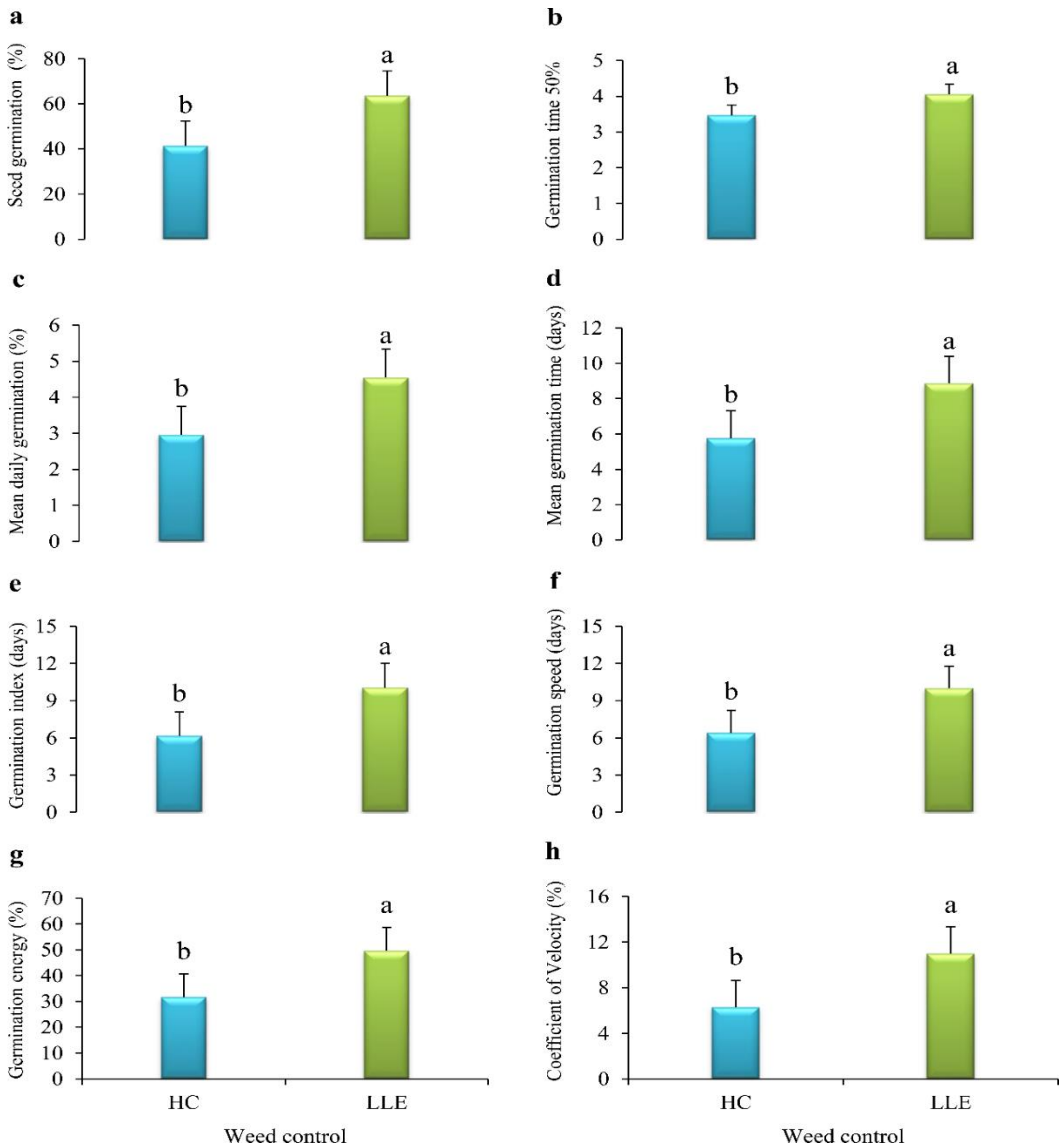

Figure (4): Effective herbicidal and allelopathic weed controls on the seed variables. According to Duncan's test, different letters indicate a statistical difference ($p \leq 0.05$) between treatments, and error bars represent standard deviations (n=3).

Responses of Seeds to Combined Herbicidal and Allelopathic Concentrations

The data in this study, implementing Analysis of Variance (ANOVA) for seed characteristics of both ARG and celery plants (Table 2), revealed significant differences among the studied variables at a significance level of $p \leq 0.05$ across diverse herbicidal and allelopathic treatments under-applied HC and LLE in varying concentrations. The most effective action for both application and inhibition of germination of ARG to zero was observed across all HC concentrations (0, 1, 2, 3, 4 mL/L). This indicates that all doses, whether below or above the recommended (1.5 mL/L) levels, impact seed weed control effectively. The seed attributes affected by reduced germination include seed germination, mean daily germination, mean germination time, germination index, germination speed, germination energy, germination time %50, and coefficient of velocity.

Table (2): Responses of seeds to combined herbicidal and allelopathic concentrations

Plants	Weed control	Doses	Seed germination (%)	Germination time 50%	Mean daily germination (%)	Mean germination time (days)	Germination index (days)	Germination speed (days)	Germination energy (%)	Coefficient of Velocity (%)
ARG	HC (mL/L)	0	51.67 e	2.08 d	3.69 e	8.66 e	7.51 efg	7.46 def	56.67 cde	11.55 ab
		1	0.00 f	0.00 e	0.00 f	0.00 h	0.00 h	0.00 g	0.00 h	0.00 d
		2	0.00 f	0.00 e	0.00 f	0.00 h	0.00 h	0.00 g	0.00 h	0.00 d
		3	0.00 f	0.00 e	0.00 f	0.00 h	0.00 h	0.00 g	0.00 h	0.00 d
		4	0.00 f	0.00 e	0.00 f	0.00 h	0.00 h	0.00 g	0.00 h	0.00 d
	LLE (%)	0	70.67 c	2.40 d	5.05 c	8.75 e	11.48 cd	11.38 bcd	79.33 a	7.65 c
		2.5	85.33 b	2.40 d	6.10 b	8.60 e	7.33 efg	7.33 def	53.33 cdef	11.62 ab
		5	49.33 e	2.13 d	3.52 e	8.18 f	5.85 fg	5.85 ef	36.67 fg	12.25 ab
		10	51.67 e	2.63 d	3.69 e	8.20 f	7.16 efg	7.16 ef	40.00 ef	12.20 ab
		15	63.00 d	2.22 d	4.50 d	7.71 g	5.06 g	5.06 f	23.33 g	12.98 a
Celery	HC (mL/L)	0	89.33 a	5.98 abc	6.38 a	9.64 bc	16.06 a	16.06 a	55.00 cde	10.37 abc
		1	61.67 d	7.16 a	4.41 d	10.01 a	9.74 cde	9.74 cde	36.67 fg	9.99 bc
		2	50.00 e	6.18 abc	3.57 e	9.69 abc	8.77 def	12.01 bc	41.67 ef	10.32 abc
		3	89.33 a	6.33 abc	6.38 a	9.73 abc	9.80 cde	9.38 cde	73.33 ab	10.28 abc
		4	71.67 c	6.96 ab	5.12 c	9.86 ab	9.56 cde	9.37 cde	53.33 cdef	10.14 abc
	LLE (%)	0	60.00 d	5.74 bc	4.29 d	9.52 bc	12.08 bcd	12.08 bc	43.33 def	10.51 abc
		2.5	60.00 d	6.01 abc	4.29 d	9.68 abc	11.77 cd	11.58 bc	50.00 cdef	10.34 abc
		5	73.33 c	5.92 abc	5.24 c	9.46 c	15.16 ab	15.16 ab	61.67 bc	10.58 ab
		10	69.33 c	5.67 bc	4.95 c	9.37 cd	12.73 bc	12.50 abc	60.00 bcd	10.67 ab
		15	53.33 e	5.38 c	3.81 e	9.11 d	11.86 cd	11.86 bc	48.33 cdef	10.99 ab

*ARG: annual ryegrass, HC: Herbicide, LLE: Laurel leaf extract

In contrast, HC applications significantly influenced celery seed parameters. The maximum values for seed germination (89.33%), mean daily germination (6.38%), germination index (16.06 days), and germination speed (16.06 days) were recorded in untreated seeds under HC concentrations. This suggests that the HC positively affects these parameters when applied to celery seeds. Moreover, the findings proved that HC significantly increased the mean germination time and germination time %50 of celery seeds compared to other treatments. Accordingly, the highest values (10.01 days and 7.15, respectively) were obtained from the plants treated with HC at 1 mL/L. The data display an increasing trend in germination energy (79.33%) for ARG seeds treated with untreated allelopathic doses of LLE applications. By increasing the percentage doses of allelopathic applications of LLR up to the maximum level (15%), the coefficient of velocity percentage of ARG was stimulated to the highest value (12.98%) among all others.

Multivariate Analysis

A Pearson correlation analysis was performed to investigate the relationships between the variables in this study (Figure 5). The results showed a strong positive relationship between SG and MDG (r^2 = 1.00, $p < 0.0001$), MGT (r^2 = 0.99, p = 0.010), GE (r^2 = 0.99, p = 0.014), and CV (r^2 = 0.97, p = 0.028). The variable GT exhibited an adequate positive correlation with the variable GS (r^2 = 0.95, p = 0.048). There was a strong and substantial positive correlation between MDG and MGT (r^2 = 0.99, p = 0.010), GE (r^2 = 0.99, p = 0.014), and CV (r^2 = 0.97, p = 0.028). The association between MGT and GE was found to be strong (r^2 = 1.00, p = 0.002), as well as the correlation between MGT and CV (r^2 = 0.96, p = 0.039). A strong positive relationship was found between GI and GS, with a high level of statistical significance (r^2 = 1.00, p = 0.001). The findings demonstrated a significant association between GE and CV, with a coefficient of determination (r^2) of 0.99 and a p-value of 0.029.

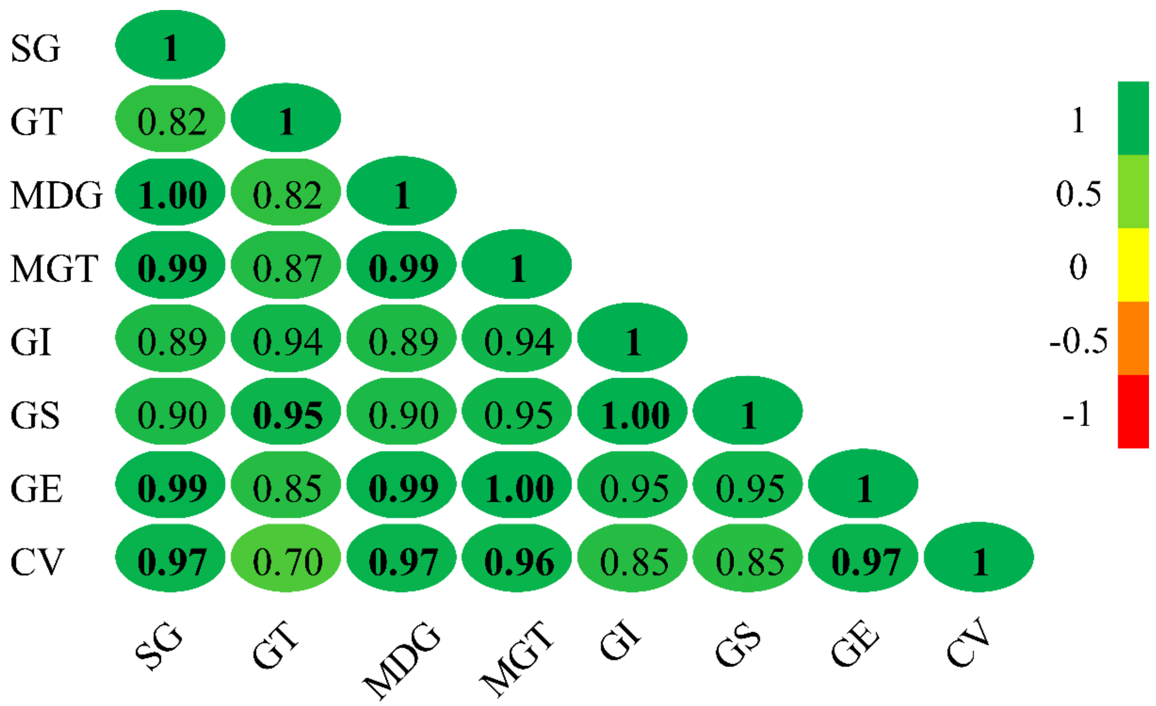

Figure (5): A Pearson's correlation analysis was conducted on all variables being investigated, with a significance level set at $p \leq 0.05$. This analysis provided data regarding the direction and degree of the correlations. SG: Seed germination, GT: Germination time 50%, MDG: Mean daily germination, MGT: Mean germination time, GI: Germination index, GS: Germination speed, GE: Germination energy, CV: Coefficient of Velocity.

In this study, we investigated the effects of herbicidal (HC) and allelopathic (LLE) actions at different dosage treatments on the seed germination and growth of ARG weed plants and celery vegetable crops. Comprehensive weed management is crucial for optimizing agricultural productivity and ensuring long-term sustainability. Weeds have been present since the early days of agriculture, and farmers recognized that these uncultivated plant species interfered with the growth of their intended crops [23, 24]. Weeds are a significant restriction on food production in agricultural systems worldwide. Weeds affect crop growth and reduce productivity by competing with crops for essential resources such as water, sunlight, nutrients, and space. Additionally, weeds can negatively impact the quality of the harvested product through allelopathy [25]. Moreover, weeds decrease land value and interfere with water management [26, 27]. Weed management in vegetable crops is crucial for maintaining high yields and quality produce. Traditionally, chemical herbicides have been the primary method for controlling weed growth, offering quick and effective results [28, 29]. However, the over-reliance on herbicides has raised concerns due to potential environmental hazards, the development of herbicide-resistant weed species, and negative impacts on non-target organisms [30]. Herbicides are designed to target specific plant species, primarily uncultivated plants or weeds, that compete with main crops [31, 32]. These chemical agents function by interfering with critical biological processes unique to weeds, such as amino acid synthesis, photosynthesis, or cell division [33]. The specificity of herbicides allows them to selectively eliminate or suppress weed growth without causing significant harm to the main crops [34, 35].

According to the data (Figure 3 and Table 2), the application of herbicides significantly impacted annual ryegrass (ARG) weed plants, notably decreasing their seed germination rates. In contrast, when the same herbicide doses were applied to celery vegetable crops, there was minimal effect on their seed attributes. This differential response highlights the selective action of the herbicide, effectively targeting the weed species while sparing the crop plants. Such specificity is crucial for effective weed management in agricultural systems, ensuring that the main crops, like celery, remain largely unaffected by herbicide treatments aimed at controlling weed populations. This selective efficacy underscores the importance of choosing the right herbicide and dosage to optimize weed control while maintaining crop health and productivity. Plant extracts significantly enhance plant performance. These natural compounds offer a sustainable and eco-friendly alternative to synthetic chemicals, benefiting both crops and the environment [36]. As a sustainable alternative, allelopathy or allelochemical, the natural process by which certain plants release biochemicals or secondary metabolism to inhibit the growth of surrounding weeds, has garnered significant interest [17]. Utilizing allelopathic plants or their residues can reduce weed pressure in vegetable crops, thereby decreasing dependence on synthetic herbicides (Figure 3). It was supported by [37], who explained that The reduction in seed germination, shoot, and root length, as well as shoot and root dry matter of the weed plants, can result from the influence of allelopathic compounds, such as phenolic acids present in *Laurus nobilis* shoot extracts. The compounds found in Laurel plants play a crucial role in biological weed management through their allelopathic effects [38].

Moreover, herbicides have a more pronounced effect on weed plants compared to allelopathic actions (Figure 4). While allelopathy involves the natural production of biochemicals by certain plants to inhibit the growth of nearby competitors, its impact is often slower and less potent. In contrast, herbicides are specifically engineered to target and eliminate weeds rapidly and effectively. These chemicals disrupt vital physiological processes within the weed plants, leading to their swift demise. Consequently, in agricultural and horticultural settings, herbicides are frequently preferred for their reliability and efficiency in weed management, overshadowing the subtler and more variable effects of allelopathy. It aligns with what researchers have documented [39]. Integrating judicious herbicide with allelopathic strategies can enhance weed control efficacy, promote biodiversity, and contribute to more sustainable agricultural practices [40]. When applying herbicides to weeds, it is important to optimize doses to avoid affecting beneficial soil microbes and causing environmental impact. Also, the focus on the use of allelochemicals against weeds will be a wise decision only if all other aspects are thoroughly explored for sustainable agricultural production.

In this study, we investigated the effects of herbicidal and allelopathic actions on weed plants and vegetable crops to determine optimal doses for inhibiting seed germination of ARG and promoting celery seed growth. Our data showed that HC exhibited superior allelopathic effects on ARG weed plants, significantly reducing seed germination. Notably, all HC concentrations completely inhibited ARG seed germination, resulting in a zero-germination rate followed by LLE decreased germination rate of ARG seed. In contrast, untreated celery seeds demonstrated higher responsiveness across various seed variables. These findings underscore the potential of allelopathic doses in agricultural practices, highlighting the importance of further research to refine these methods for improved crop production and weed management. This study contributes to advancing commercial agriculture by identifying effective allelopathic strategies to enhance crop yield and health.

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